

Exposure of Rats to 425-MHz (CW)
Radiofrequency Radiation: Effects on Lymphocytes

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Exposure of Rats to 425-MHz (CW) Radiofrequency Radiation: Effects on Lymphocytes



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ABSTRACT

Four experiments were performed in which six pregnant rats were exposed from day 12 of pregnancy to parturition, for 4 hours a day in a temperature-controlled environment, to 425-MHz (CW) radiation, using a multimode rectangular strip transmission line. Four male pups born to each dam were subsequently irradiated under the same RF exposure condition for 20–21 days of age (2 pups) and 40–41 days of age (2 pups). Specific absorption rates (SARs) for rats of different ages were determined by twin-well calorimetry as well as from calculations of power measurements of incident, reflected, and transmitted energy. Values of SARs between 3.1 and 6.7 mW/g were obtained for rats so exposed at 425 MHz. At selected times, rats were weighed to determine if the irradiation affected growth. Two rats from each litter (4 pups) were euthanized at 20–21 and two at 40–41 days of age and blood was obtained for complete blood counts. The *in vitro* blastogenic response of blood and lymph-node lymphocytes was measured by ³H-thymidine incorporation into DNA following stimulation of cells with T- or B-lymphocyte mitogens. No difference was observed in the weights of irradiated compared with sham-irradiated rats. No consistent change in the peripheral blood picture was observed between irradiated and sham-irradiated rats. Significant increases in the response of lymph-node but not of blood lymphocytes from irradiated rats following stimulation with mitogens was observed in two of four experiments. These changes were observed for both T- and B-lymphocytes. In another experiment at the same frequency, six pregnant rats were irradiated for 16 hours daily from day 6 through day 19 of pregnancy. The pups born to these dams were not subsequently irradiated. These rats, born to irradiated dams, showed a similar increased response of node but not of blood lymphocytes to T-cell mitogens at 42 days of age. These results indicate that exposure to 425-MHz microwave radiation, under the conditions described, may lead to increased responsiveness of node lymphocytes to *in vitro* stimulation by mitogen.

INTRODUCTION

Reports from several laboratories indicate that the functional activity of lymphocytes may be changed following exposure to non-ionizing electromagnetic radiation. Czerski [1975] reported that mice irradiated at 2950 MHz had significantly greater numbers of antibody-producing cells and higher serum-hemagglutination titers following immunization with sheep erythrocytes. Prince et al. [1972] found an increased sensitivity of lymphocytes to phytohemagglutinin stimulation after monkeys were exposed to radiation at 10 to 27 MHz. *In vitro* irradiation of human lymphocytes by 2950-MHz pulsed microwaves gave rise to a 'spontaneous lymphoblastoid transformation' and to an increased mitotic index [Stodolnik-Baranska, 1967]. Wiktor-Jedrzejczak et al. [1977] reported that irradiation of mice at 2450 MHz induces an increase in the number of complement-receptor B-lymphocytes in the spleen of these animals. Huang et al. [1977] reported increased transformation to lymphoblasts in cultures of blood lymphocytes from hamsters exposed to 2450 MHz. All of these studies indicate that the functional activity of lymphocytes may be changed by exposure to RF radiation. Studies in our laboratory indicate that long-term irradiation at 2450 MHz of rats *in utero* and during early post-natal life may affect the *in vitro* mitogen-stimulated response of lymphocytes in young animals [Smialowicz et al., 1979]. In the present study we have employed a similar protocol in an attempt to determine if chronic low-level exposure to 425-MHz (CW) RF radiation has a comparable effect on lymphocytes.

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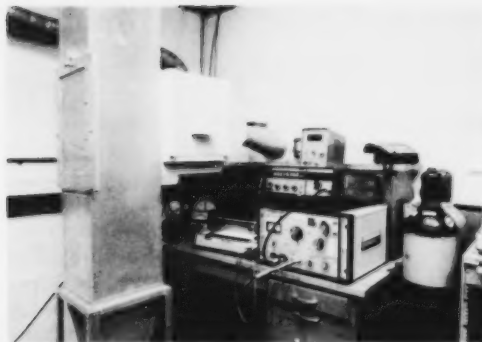


Figure 1 Exposure facility for 425-MHz irradiation. Sham-irradiated controls were placed in foamed polystyrene chamber to right of the transmission line. Irradiated rats were placed on either side of divided transmission line. Temperature in control chamber was monitored by a thermocouple probe. Air at constant temperature was passed through entire system during exposures.

In the present study we performed four experiments in which time-bred rats and their pups were exposed to 425-MHz (CW) RF radiation. In a fifth experiment the dams but not their pups were irradiated. The blastogenic and proliferative capacity of thymus-derived (T) and bursa-equivalent (B) lymphocytes was measured by the incorporation of ^3H -thymidine into nucleic acid, [Anderson et al., 1972; Newlin and Gasser, 1973; Stobo and Paul, 1973; Strong et al., 1974; and Williams et al., 1973]. The T-lymphocyte mitogens employed were phytohemagglutinin and concanavalin A; and the B-lymphocyte mitogens were lipopolysaccharide and purified protein derivative of tuberculin. Pokeweed mitogen, which stimulates both T- and B-lymphocytes, was used in some experiments.

The results obtained in these studies indicate that irradiation of rats, under the conditions described, gives rise to an increased ability of lymphocytes to undergo blastic transformation following stimulation with mitogens.

MATERIALS AND METHODS

Microwave Exposure System

The exposure system (see Fig. 1) consisted of a rectangular strip transmission line [Crawford, 1974] designed and fabricated for EPA in-house research. The line exhibits a TEM mode 50-ohm characteristic impedance according to the design criteria of Weil [1978]. The cross-sectional dimensions of the line are as follows: width of side parallel to center conductor, $a = 33$ cm; width of side normal to center conductor, $b = 30.5$ cm; and width of center conductor, $w = 27.9$ cm. The transmission line is approximately three meters in length and was constructed from 3.2 mm aluminum sheet; the line is oriented such that its axis is vertical. The weight of the center conductor is supported by a 2.54-cm diameter fiberglass rod that is anchored on opposite sides of the line; foamed polystyrene spacers are used to maintain the proper spacing between outer shield and center conductor. Access to the interior of the line is provided by means of a hinged door. Ventilation of the interior space is provided by means of two windows made of perforated aluminum sheet located in the door and on the opposite side of the outer shield. A sham irradiation chamber constructed of foamed polystyrene was attached to the line at the location of the latter window. A one-pass environmental control system fed conditioned air serially through the access door into the room. Air was pre-cooled and then warmed to the required temperature using an electric space heater that was regulated by means of a proportional temperature controller, the sensor of which was located in the sham chamber.

The system was excited using a model AIL 135 RF power generator (AILTECH, Farmingdale, N.Y.) capable of supplying up to 80 W output. Incident, reflected and transmitted powers were monitored using three NARDA model 3000 20-dB directional couplers and three HP 435A power

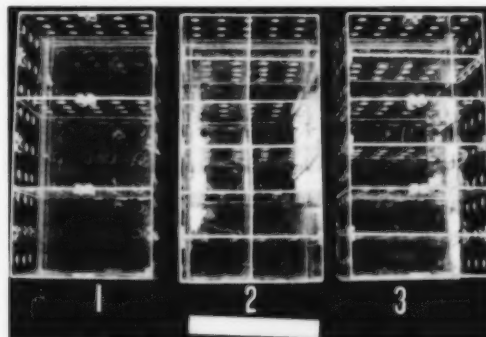


Figure 2 Vented acrylic cages used to hold irradiated and sham-irradiated rats. (1) Three pregnant rats were originally and individually placed in each section of cage. (2) At delivery litters were normalized to four male pups, which were individually placed in one of 12 sections of the cage. (3) At 20 to 21 days of age, two pups were euthanized and remaining two were placed individually in one of six sections of the cage. Feces and urine were collected in tray beneath the cage.

meters (Hewlett-Packard Co., Palo Alto, Calif.). A model S2-02N double stub tuner was used to match the line input to the power source; the line's output port was terminated in a model TB-7MN high power 50 Ω load (both components supplied by Microlab/FXR, Livingston, N.J.). A model 235-5 circulator (Electromagnetic Sciences, Atlanta, Ga.) was also used to help protect the RF power source in the event of any high mismatch conditions.

An operating frequency of 425 MHz, was selected for this system, primarily because the first higher order mode had been originally predicted to occur at about 450 MHz, using the criteria suggested by Crawford [1974]. Consequently, the unloaded transmission line was assumed to be operating in the basic TEM mode at this frequency. After the conclusion of the irradiation experiments described herein, subsequent information revealed that this assumption was incorrect because the first higher order mode (TE_{01}) begins to propagate in this structure at about 280 MHz and not 450 MHz [Gruner, 1979]. Measurements made of the cross-sectional distribution of internal fields in the transmission line appeared to confirm the presence of the TE_{01} mode at 425 MHz [Weil, Joines, and Kinn, 1981]. Consequently, animal exposures were, in fact, conducted under multimodal conditions rather than TEM mode. The existence of higher order modes creates a greater nonuniformity of the fields within the line. Also, the propagating wave within the line cannot be considered to be plane-wave equivalent and, consequently, the power density concept is not applicable in the exposure conditions. For this reason, the incident exposure rate is defined only in terms of the forward power being fed into the transmission line.

Animal Exposures

Six time-bred pregnant rats (COBS CD F/Crl BR, Charles River Labs, Wilmington, Mass.) were housed in two acrylic cages (Fig. 2) that were divided into three compartments each and were located on either side of the center conductor. Four separate experiments were performed in which six pregnant rats were simultaneously exposed, starting on day 12 of pregnancy. The temperature was maintained at 22°C during irradiation of the dams. At delivery, litters born to the dams were normalized to four male pups. The four pups were then placed in the subdivided section of the cage originally occupied by the dam (see Fig. 2). The temperature was maintained at 26.7°C during the first 14 days *post-partum* and was then lowered to 22°C for the duration of irradiation exposures. The air flow within the sham and exposure chamber was maintained at 1.3 m³/min. In experiments 1, 2, and 3 the exposure of the dams during pregnancy and the subsequent exposure of the pups occurred at a forward power of 20 W. In the fourth experiment the irradiated dams were inadvertently exposed at a lower forward power (i.e., 6.6 W). However, the postnatal irradiation of their pups occurred at 20 W.

All irradiation exposures were conducted daily for 4 hours in the morning. An identical number of control rats were sham-irradiated in the temperature-controlled sham chamber during the RF irradiation of the experimental rats (Fig. 1). Control and irradiated rats were handled in a similar fashion throughout the experiments and were housed in adjacent cages in the animal-care facility. All rats had access to food and water *ad libitum* prior to and after irradiation. At 20–21 days of age, two pups from each litter were euthanized, the remaining two rats per litter were irradiated for 20 more days and were euthanized at 40–41 days. All rats were euthanized between the 20th and 24th hour following the last exposure to microwaves.

In a fifth experiment, pregnant rats were exposed for 16 hours each night from day 6 to day 19 of gestation to 425-MHz (CW) RF radiation at a forward power of 20 W. All rats had access to food and water *ad libitum* prior to and after irradiation. After birth, the litters were normalized to four male pups each and the pups were kept in the animal facility with no further irradiations. At 22 and 42 days of age, two pups from each litter were euthanized.

Determination of Body Mass

At selected times during the irradiation process, rats were weighed so that the growth of the pups could be measured. Rats were always weighed immediately prior to irradiation. The mean body mass of pups in each litter was determined, and the mean mass of litters of control and irradiated rats was evaluated by analysis of variance. Data are presented in Table 1 as the mean mass of litters ± 1 S.D.

Dosimetry

The averaged values of specific absorption rates (SARs) of individual rats were determined by twin-well calorimetry [Kinn, 1977]. Absorption measurements were made using an individual rat carcass placed in a section of a cage. All other positions were filled with live animals. The average SARs for rats located in the center position of the TE₀₁ line are shown in Table 2. SARs for animals located at the end of the line were half those in the center. The SAR values also were computed from measurements of incident, reflected, and transmitted powers. Data from the power meters were fed to an analog summing circuit that provided an analog voltage proportional to the rate of energy absorption in the transmission line. These data were fed to a minicomputer in which they were sampled continuously, digitized, and recorded. Energy absorption data were time-averaged over the 4-hour exposure period and an SAR value averaged over the number of exposed animals was derived. Average SAR values are expressed as the mean mW/g ± 1 S.D. (Table 2).

Hematology

At the times specified, rats were bled from the aorta while under ether anesthesia. Smears for differential cell counts were prepared and stained with Wright stain. Two hundred leukocytes were counted and the percentage of different leukocytes was recorded. Heparinized blood, 15 units of heparin sodium per ml of blood, was evaluated on a model ZBI-6 Coulter counter (Coulter Electronics, Inc., Hialeah, Fla.) for erythrocyte count, leukocyte count, mean cell volume of erythrocytes, hematocrit, and hemoglobin concentration. Relative and absolute cell counts are expressed as the mean ± 1 S.D. (Table 3). Data were statistically analyzed by analysis of variance. In order to prevent false positive results from the numerous analyses performed on blood, Bonferroni's Inequality was applied [Wallenstein et al., 1980; Lee et al., 1980]. This procedure is very conservative in that a *P* value of less than 0.05 normally used for significance at the 5% level was divided by 8, the total number of independent analyses. Therefore a *P* value of less than 0.006 was the significant level for these data.

In Vitro Lymphocyte Response

Blood was collected from the aorta at the time of euthanizing and was placed in sterile tubes containing heparin sodium (15 units/ml blood). Lymphocytes were separated from whole blood by the method of Boyum [1968] using lymphocyte separation medium (LSM, Bionetics, Kensington, Md.). The separated mononuclear cells were washed twice with RPMI-1640 medium (Grand Island Biological Co., Grand Island, N.Y.) containing 100 units/ml penicillin, 100 mg/ml streptomycin, and 10% heat-inactivated fetal calf serum. The cells were counted on a hemocytometer using trypan blue and were suspended in complete RPMI medium to 2×10^6 viable cells/ml. Where indicated, lymphocytes were cultured in whole blood cultures according to Pauly et al. [1973]. Lymph-node lymphocytes were obtained by mincing axillary, inguinal, and cervical lymph nodes in complete RPMI

medium. Cell suspensions were washed twice and resuspended in complete medium to 2×10^6 viable cells/ml.

Blood and node lymphocytes were cultured in sterile, flat-bottom microtiter plates (Falcon Plastics, Oxnard, Calif.). Triplicate or quadruplicate cultures containing 2×10^5 cells in a 0.2 ml volume of either complete medium or complete medium with mitogen were prepared for lymphocytes from individual control and irradiated rats. Several concentrations of one or more of the following mitogens, diluted in RPMI medium, were used to stimulate lymphocytes in culture: purified phytohemagglutinin (PHA, Burroughs Wellcome Co., R.T.P., N.C.), concanavalin A (Con A, Miles Laboratories, Inc., Kankakee, Ill.), bacterial lipopolysaccharide of *Escherichia coli* 0128:B12 (LPS, Difco Lab., Detroit, Mich.), pokeweed mitogen (PWM, Grand Island Biological Co., Grand Island, N.Y.), and purified protein derivative of tuberculin (PPD, Parke-Davis and Co., Detroit, Mich.). Cultures were incubated for 72 hours in a humidified atmosphere of 95% air and 5% CO₂ at 37°C. Twenty-four hours before harvesting, cultures were pulsed with 0.5 μ Ci of methyl-³H-thymidine (³H-TdR, specific activity 6.7 Ci/mM, New England Nuclear, Boston, Mass.). The cells were collected onto glass-fiber filter paper strips No. 934AH, Whatman, Inc., Clifton, N.J.) with a MASH II automated harvester (Microbiological Associates, Bethesda, Md.), and DNA was precipitated with ice-cold 5% trichloroacetic acid. Air-dried samples were transferred to scintillation vials containing Omnifluor-toluene (New England Nuclear) and then counted in a tri-carb liquid scintillation counter (Packard Instrument Co., Downers Grove, Ill.).

Considerable variability in the lymphoproliferative response among rats has been observed in this laboratory. In order to minimize the variability, we adhered to strict lymphocyte culture procedures and tested age- and sex-matched controls at the same time as experimental subjects. Also, data collected as counts per minute (CPM) were log₁₀ transformed to better satisfy the assumption of normality of variance. Nevertheless, considerable differences in response were observed for some of the rats. In order to further reduce the effect of such variability on the results, two very conservative statistical tests for outliers in the data were done prior to analysis. The first test [Bliss et al., 1956] was used to detect outlying replicate values, and the level of significance was set at $\alpha = 0.001$. The second test [Dixon, 1953] was used to detect outlying responses by individual rats ($\alpha = 0.005$). A total of 28 data points out of a total of 2856 were found to be significantly different and were not included in the final analysis of response. The responses of lymphocytes from irradiated and from control rats were compared for each concentration of mitogen. Results presented in the text are given as the mean plus or minus one standard error of the mean (± 1 S.E.) of nontransformed data. Log-transformed data were evaluated by analysis of variance.

RESULTS

Growth

Table 1 shows data on the growth of rats. There was no difference in the mean masses of litters between six groups of control and irradiated rats.

Table 1 Mean masses of litters of irradiated and sham-irradiated rats (Experiment No. 1)

Post-Natal Age in Days	Mean Mass in Grams of Normalized Litters ($\bar{X}_g \pm SD$)		p ^b
	Control (6) ^a	Irradiated (6)	
1	7.50 $\pm$ 0.34	7.80 $\pm$ 0.75	N.S. ^c
5	12.2 $\pm$ 1.0	12.0 $\pm$ 1.0	N.S.
7	16.2 $\pm$ 2.2	16.7 $\pm$ 0.97	N.S.
10	23.9 $\pm$ 3.1	25.6 $\pm$ 1.8	N.S.
21	56.9 $\pm$ 4.1	59.9 $\pm$ 4.1	N.S.
31	114 $\pm$ 9.9	110 $\pm$ 13.4	N.S.
39	185 $\pm$ 14.0	178 $\pm$ 10.8	N.S.

^aNo. of litters

^bProbability

^cNot significant ($P > 0.05$)

Table 2 Average specific absorption rates (SARs) of rats exposed at a forward power of 20 W

AGE (DAYS)	RANGE OF BODY MASSES (g)	AVERAGE SPECIFIC ABSORPTION RATES	
		($\bar{X}$ mW/g $\pm$ SD) ^a	($\bar{X}$ mW/g $\pm$ SD) ^b
1-5	6-12	4.1 $\pm$ 0.7 (8) ^c	6.7 $\pm$ 1.1 (24) ^d
8-20	15-60	3.1 $\pm$ 0.9 (37)	3.5 $\pm$ 0.2 (24)
25-35	90-140	4.5 $\pm$ 1.2 (14)	4.1 $\pm$ 0.1 (12)
PREGNANT RATS	270-350	6.1 $\pm$ 0.8 (12)	4.7 $\pm$ 0.4 (6)

^aSAR values derived by twin-well calorimetry.^bSAR values derived from incident, reflected and transmitted powers as time-averaged over exposure period and averaged over number of animals exposed.^cNumber of determinations.^dNumber of animals exposed simultaneously.*Dosimetry*

Table 2 gives the SAR values for six dams and their litters. There is relatively good agreement between the SAR values obtained by twin-well calorimetry and extrapolated values based on power-meter readings. Colonic temperatures were not measured during these experiments owing to the large numbers of animals. However, colonic temperatures in a sample of pregnant rats showed no increase following exposure.

Table 3 Peripheral blood counts of irradiated (20 W) rats

Exp. No.	Age (days)	Animals (N) ^a	WBC/mm ³ ($\bar{X} \pm$ SD)	% Lymphocytes ($\bar{X} \pm$ SD)	Lymphocytes/mm ³ ($\bar{X} \pm$ SD)	% PMN ($\bar{X} \pm$ SD)	PMN/mm ³ ($\bar{X} \pm$ SD)
1	40-41	Control (6)	8,400 $\pm$ 1,800	75 $\pm$ 6	6,100 $\pm$ 1,400	13 $\pm$ 6	1,070 $\pm$ 500
		Irradiated (6)	7,850 $\pm$ 1,200	77 $\pm$ 7	6,300 $\pm$ 1,600	13 $\pm$ 5	980 $\pm$ 340
2	20-21	Control (12)	4,200 $\pm$ 1,050	74 $\pm$ 9	3,100 $\pm$ 900	22 $\pm$ 9	950 $\pm$ 480
		Irradiated (11)	4,000 $\pm$ 1,050	66 $\pm$ 7	2,600 $\pm$ 650	26 $\pm$ 6	1,050 $\pm$ 440
	40-41	Control (12)	8,200 $\pm$ 2,900	83 $\pm$ 5	6,800 $\pm$ 2,600	14 $\pm$ 4	1,100 $\pm$ 400
		Irradiated (12)	8,200 $\pm$ 2,000	87 $\pm$ 3	7,100 $\pm$ 1,800	9 $\pm$ 3 ^b	690 $\pm$ 260
3	20-21	Control (10)	5,700 $\pm$ 3,360	80 $\pm$ 9	4,700 $\pm$ 2,900	17 $\pm$ 8	880 $\pm$ 560
		Irradiated (11)	8,300 $\pm$ 4,200	76 $\pm$ 10	6,300 $\pm$ 3,300	23 $\pm$ 10	1,900 $\pm$ 1,300
	40-41	Control (12)	7,900 $\pm$ 2,000	85 $\pm$ 4	6,700 $\pm$ 1,700	13 $\pm$ 4	1,030 $\pm$ 490
		Irradiated (11)	8,700 $\pm$ 2,300	83 $\pm$ 4	7,300 $\pm$ 2,100	16 $\pm$ 4	1,330 $\pm$ 460
4 ^d	20-21	Control (12)	N.D. ^c	77 $\pm$ 6	N.D.	21 $\pm$ 6	N.D.
		Irradiated (12)	N.D.	78 $\pm$ 5	N.D.	19 $\pm$ 4	N.D.
	40-41	Control (12)	7,900 $\pm$ 2,600	76 $\pm$ 8	6,000 $\pm$ 1,600	21 $\pm$ 8	1,800 $\pm$ 1,300
		Irradiated (12)	7,000 $\pm$ 1,380	84 $\pm$ 10	5,900 $\pm$ 1,300	12 $\pm$ 8	900 $\pm$ 700

^aNo. of animals studied.^b $p < 0.005$, analysis of variance applying Bonferroni's Inequality.^cNot done.^dIrradiated dams exposed to 6.6W and pups exposed to 20W.

Table 4 Mitogen-stimulated response of lymphocytes from irradiated (20 W) rats (Experiments No. 1 and 2)

Exp. No.	Age (days)	Source of lymphocytes	Mitogen (μ g/culture)	^3H -TdR Uptake ($\bar{X}$ CPM $\pm$ SEM)		p^b
				Control (N) ^a	Irradiated (N)	
1	40-41	Lymph nodes	0	700 $\pm$ 190 (6)	760 $\pm$ 100 (6)	N.S. ^c
			PHA - 0.13	75,200 $\pm$ 4,600	90,100 $\pm$ 3,400	< 0.05
			PHA - 0.25	69,800 $\pm$ 10,800	100,300 $\pm$ 2,300	< 0.05
	40-41	Blood ^d	0	170 $\pm$ 30 (6)	140 $\pm$ 30 (6)	N.S.
			PHA - 0.5	13,600 $\pm$ 2,500	17,100 $\pm$ 3,600	N.S.
2	20-21	Lymph nodes	0	75 $\pm$ 10 (12)	100 $\pm$ 20 (11)	N.S.
			PHA - 0.05	7,270 $\pm$ 1,500	23,800 $\pm$ 6,600	< 0.05
			PHA - 0.25	71,700 $\pm$ 6,300	89,600 $\pm$ 6,100	< 0.10
	40-41	Lymph nodes	0	200 $\pm$ 90 (12)	240 $\pm$ 100 (12)	N.S.
			PHA - 0.25	24,300 $\pm$ 6,400	56,400 $\pm$ 8,600	< 0.01
			ConA - 0.5	41,600 $\pm$ 9,400	87,000 $\pm$ 11,800	< 0.05
			PWM - 25	200 $\pm$ 40	1,060 $\pm$ 350	< 0.001
			LPS - 0.5	680 $\pm$ 210	2,980 $\pm$ 1,240	< 0.05
	40-41	Blood	0	380 $\pm$ 90	250 $\pm$ 20	N.S.
			PHA - 0.5	22,400 $\pm$ 3,700	24,200 $\pm$ 4,400	N.S.
			ConA - 1.0	73,700 $\pm$ 15,300	91,200 $\pm$ 6,000	N.S.

^aNo. of animals studied.^b p s based on analysis of variance of log-transformed CPM.^cNot significant^dWhole blood cultures.

Hematology

Table 3 shows data on leukocyte counts for rats from the first four experiments. No consistent significant changes in the leukocyte counts of rats in any of the experiments were observed. In experiments 2 and 4 a relative increase in the percentage of lymphocytes, as well as a relative and an absolute neutropenia, was observed in the 40-41-day old irradiated rats. These differences, however, were not statistically significant when Bonferroni's Inequality was applied. No differences in erythrocyte counts, or in hematocrit or hemoglobin concentrations were observed between control and irradiated rats in any of the four experiments.

Lymphocyte Response

In all experiments, at least three concentrations of mitogen were used so that a dose-response function for mitogenic stimulation of lymphocytes from individual rats could be obtained. The data presented are responses (a) at optimal concentrations of mitogens, and (b) at concentrations that are of significance in evaluating lymphocyte responsiveness.

In the first two experiments, a consistently significant ($P < 0.05$) increase in the mitogen-stimulated response of lymph-node lymphocytes of irradiated rats was observed (Table 4). However, no change in the blastogenic response of peripheral-blood lymphocytes was observed from these irradiated rats. Lymphocytes from microwave exposed rats in experiment 2 displayed a significantly greater uptake of ^3H -TdR as compared with controls when stimulated with either T- or B-cell mitogens. There was no difference in the response of unstimulated lymphocytes from control and irradiated rats in these experiments. However, in experiment 3, these results were not corroborated (Table 5). The blood lymphocyte response of 40-41-day old irradiated rats displayed an increased response to stimulation by PHA as compared with controls in experiments 3 and 4.

Table 5 Mitogen-stimulated response of lymphocytes from rats irradiated at 20 W (Experiment No. 3). (Experiment No. 4: Dams were irradiated at a forward power of 6.6 W and pups were exposed at 20 W)

Exp. No.	Age (days)	Source of lymphocytes	Mitogen (μ g/culture)	3H-TdR Uptake ($\bar{X}$ CPM $\pm$ SEM)		P
				Control (N)	Irradiated (N)	
3	20 - 21	Lymph nodes	0	190 $\pm$ 40 (12)	230 $\pm$ 50 (12)	N. S.
			PHA - 0.25	73,200 $\pm$ 2,900	67,500 $\pm$ 4,500	N. S.
	40 - 41	Lymph nodes	0	95 $\pm$ 10 (12)	90 $\pm$ 10 (11)	N. S.
			PHA - 0.25	26,900 $\pm$ 2,800	22,500 $\pm$ 4,400	N. S.
			ConA - 0.50	68,900 $\pm$ 8,100	53,400 $\pm$ 9,700	N. S.
			PWM - 25	1,900 $\pm$ 300	1,300 $\pm$ 300	N. S.
			LPS - 0.5	1,500 $\pm$ 280	1,300 $\pm$ 300	N. S.
			PPD - 25	660 $\pm$ 120	620 $\pm$ 90	N. S.
	40 - 41	Blood	0	430 $\pm$ 160	600 $\pm$ 110	N. S.
			PHA - 0.25	33,300 $\pm$ 6,400	50,300 $\pm$ 2,500	< 0.05
			PHA - 1.0	61,600 $\pm$ 7,100	43,500 $\pm$ 14,000	N. S.
4 ^a	20 - 21	Lymph nodes	0	250 $\pm$ 50 (12)	170 $\pm$ 30 (12)	N.S.
			PHA - 0.25	74,400 $\pm$ 3,600	66,200 $\pm$ 6,300	N.S.
			ConA - 0.5	63,500 $\pm$ 5,400	62,600 $\pm$ 7,800	N.S.
	40 - 41	Lymph nodes	0	80 $\pm$ 10 (12)	90 $\pm$ 20 (12)	N.S.
			PHA - 0.25	52,200 $\pm$ 5,000	53,500 $\pm$ 9,300	N.S.
			ConA - 0.25	71,000 $\pm$ 10,700	71,700 $\pm$ 14,800	N.S.
			LPS - 0.5	2,400 $\pm$ 730	2,500 $\pm$ 940	N.S.
	40 - 41	Blood	0	600 $\pm$ 150	510 $\pm$ 60	N.S.
			PHA - 0.5	38,200 $\pm$ 5,600	62,200 $\pm$ 9,600	< 0.10

^aIrradiated dams exposed at 6.6W and pups exposed at 20W.

In experiment 5 the irradiation of dams for 16 hours a day (from 1600 to 0800 hours) from days 6 to 19 of gestation produced no changes in the hematologic pictures of their pups at 22 and 42 days of postnatal age (Table 6). However, Table 7 shows that node lymphocytes from 42-day old rats born to irradiated dams incorporated significantly greater amounts of ³H-TdR than controls following stimulation with PHA and Con A. This increased responsiveness is similar to that observed in rats irradiated both pre- and postnatally (Table 4).

Table 6 Peripheral blood counts of rats only irradiated *in utero* at 20 W

Age (days)	Animals	WBC/mm ³ ($\bar{X}$ $\pm$ SD)	% Lymphocytes ($\bar{X}$ $\pm$ SD)	Lymphocytes/mm ³ ($\bar{X}$ $\pm$ SD)	% PMN ($\bar{X}$ $\pm$ SD)	PMN/mm ³ ($\bar{X}$ $\pm$ SD)
22	Control (8)	5,600 $\pm$ 2,700	83 $\pm$ 7	4,500 $\pm$ 1,800	15 $\pm$ 6	1,000 $\pm$ 900
	Irradiated (8)	5,100 $\pm$ 1,600	80 $\pm$ 12	4,000 $\pm$ 1,100	19 $\pm$ 12	1,100 $\pm$ 1,000
42	Control (8)	9,300 $\pm$ 1,800	86 $\pm$ 5	8,100 $\pm$ 1,700	12 $\pm$ 6	1,100 $\pm$ 500
	Irradiated (8)	9,700 $\pm$ 2,400	84 $\pm$ 7	8,300 $\pm$ 2,600	14 $\pm$ 7	1,300 $\pm$ 400

Table 7 Mitogen-stimulated response of lymphocytes from rats only irradiated *in utero* at 20 W

Age (days)	Source of lymphocytes	Mitogen (μ g/culture)	3H-TdR Uptake ($\bar{X}$ CPM $\pm$ SEM)		P
			Control (N)	Irradiated (N)	
22	Lymph nodes	0	470 $\pm$ 100 (8)	320 $\pm$ 70 (8)	N. S.
		PHA - 0.25	66,000 $\pm$ 2,900	58,000 $\pm$ 3,200	N. S.
42	Lymph nodes	0	900 $\pm$ 600	430 $\pm$ 110	N. S.
		PHA - 0.25	57,400 $\pm$ 7,200	76,600 $\pm$ 1,600	< 0.05
		ConA - 0.25	68,100 $\pm$ 11,700	99,800 $\pm$ 4,100	< 0.05
		PWM - 25	10,000 $\pm$ 7,000	8,700 $\pm$ 1,400	N. S.
		LPS - 0.5	5,000 $\pm$ 2,400	4,200 $\pm$ 400	N. S.
		PPD - 25	3,050 $\pm$ 1,800	2,100 $\pm$ 230	N. S.
42	Blood	0	680 $\pm$ 100	870 $\pm$ 200	N. S.
		PHA - 1.0	57,000 $\pm$ 9,100	55,500 $\pm$ 13,000	N. S.

DISCUSSION

The results of this study indicate that the perinatal exposure of rats to 425-MHz (CW) RF radiation may lead to increased mitogen-stimulated responses by lymphocytes. This finding is somewhat similar to those reported by Prince et al. [1972], Stodolnik-Baranska [1967], Czerski [1975], Baranski [1971, 1972], and Smialowicz et al. [1979], in which increases in lymphocyte metabolic or functional activity followed exposure to nonionizing electromagnetic radiation.

We have found that there is much variability in the mitogen-stimulated response of rat lymphocytes. This includes variations among animals and between experiments. To minimize the effect of this variability on the final evaluation of results, we have employed conservative statistical tests for outlying data [Bliss et al., 1956, and Dixon, 1953] and have log transformed the raw data (CPM) to better satisfy the assumption of normality of variances. Control and irradiated animal data from each individual experiment were evaluated independently of data from other experiments. That an increased response was not observed consistently for all lymphocytes cultured in every experiment may be a consequence of the variability of the assay. However, the results also indicate that we may be dealing with very subtle effects or that we are near an effect threshold level of irradiation.

While the increased response to mitogens by node lymphocytes from irradiated rats was demonstrated in only two (experiments 1 and 2) of three replicate experiments (i.e., experiments 1, 2, and 3), the results nevertheless suggest that increased lymphocyte responsiveness develops subsequent to prolonged RF radiation exposure. It is interesting to note that increased responsiveness was observed in either blood or node lymphocytes in each of experiments 1 through 4. In no instance was there any significant decrease in responsiveness.

The exposure of rats to 425-MHz RF radiation during gestation appears to be an essential factor in the development of these lymphocyte changes in the older animal. This assumption is supported by the results of this study and by experiments in which older rats (40 to 50 days of age), which were exposed to 425-MHz (CW) RF radiation at 20 W for periods up to 16 hours a day for 6 days, displayed no changes in peripheral blood or in mitogen-stimulated lymphocyte responses (Smialowicz, unpublished results).

The means by which RF radiation causes the observed changes are not known. It appears, however, that the changes in lymphocyte activity are not frequency dependent, since exposure to 425-MHz or 2450-MHz RF radiation [Smialowicz et al., 1979] gives rise to similar responses. Whether these changes in lymphocyte response reflect an alteration in the functional integrity of the exposed animal's immune system remains to be investigated.

Comparison of SAR values of RF-exposed rats to basal metabolic rates (BMRs) of rats of comparable size [Kleiber, 1975] indicates that irradiated rats in this study experience an energy accumulation greater than their BMR in some cases. The pregnant rats in the present study absorbed energy at a rate approaching the BMR for an alert, but unexcited rat [Phillips et al., 1975]. A sample

of measurements of colonic temperatures in pregnant rats failed to show increases following irradiation. The failure to demonstrate a gross thermal burden in these rats, however, does not eliminate the possibility of compensation for RF-induced heating through thermoregulatory mechanisms. In rats made hypothermic with endotoxin, significant colonic temperature increases have been observed at SAR values of 1 mW/g [Smialowicz et al., 1980].

While various physiological stresses can depress lymphocyte activity and the immune response [Thaxton and Siegel, 1973; Riley, 1976; Monjan and Collector, 1977; Folch and Waksman, 1974], prolonged exposure to stress has been shown to enhance some lymphocyte activities [Monjan and Collector, 1977]. Whether the increased response of lymphocytes from 425-MHz RF irradiated rats reported here can be explained as due to a thermally induced stress reaction requires further investigation.

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REFERENCES

- Anderson, J., G. Moller, and O. Sjorberg (1972). Selective induction of DNA synthesis in T and B lymphocytes. *Cellular Immunol.* 4, 381-393.
- Baranski, S. (1971). Effect of chronic microwave irradiation on the blood forming system of guinea pigs and rabbits. *Aerospace Med.* 42, 1196-1199.
- Baranski, S. (1972). Effect of microwaves on the reactions of the white blood cell system. *Acta Physiologica Polonica*, 23, 685-695.
- Bliss, C. I., W. G. Cochran, and J. W. Tukey (1956). A rejection criterion based upon the range. *Biometrika*, 43, 418.
- Boyum, A. (1968). Separation of leukocytes from blood and bone marrow. *Scand. J. Clin. Lab. Invest.* 21, Suppl. 97, 7.
- Crawford, M. L. (1974). Generation of standard EM fields using TEM transmission cells. *IEEE Trans. Electromagnetic Compatibility*, EMC-16, 189-195.
- Czerski, P. (1975). Microwave effects on the blood-forming system with particular reference to the lymphocyte. *Ann. N.Y. Acad. Sci.* 247, 232-241.
- Dixon, W. J. (1953). Processing data for outliers. *Biometrics*, 9, 74.
- Folch, H. and B. H. Waksman (1974). The splenic suppressor cell. I. Activity of thymus-dependent adherent cells: changes with age and stress. *J. Immunol.* 113, 137-139.
- Gruner, L. (1979). Estimating rectangular co-ax cutoff. *Microwave J.* 22, 88-92.
- Huang, A. T., M. E. Engle, J. A. Elder, J. B. Kinn, and T. R. Ward (1977). The effect of microwave radiation (2450-MHz) on the morphology and chromosomes of lymphocytes. *Radio Sci.* 12 (6S), 173-177.
- Kinn, J. B. (1977). Whole-body dosimetry of microwave radiation in small animals: the effect of body mass and exposure geometry. *Radio Science*, 12 (6S), 61-64.
- Kleiber, M. (1975). *The Fire of Life. An Introduction to Animal Energetics*, pp. 179-236. Robert E. Krieger Publishing Co., Huntington, New York.
- Lee, K. L., J. F. McNeer, C. F. Starmer, P. J. Harris, D. Phil, and R. A. Rosati (1980). Clinical judgement and statistics. *Circulation*, 61, 508-515.
- Monjan, A. A. and M. I. Collector (1977). Stress-induced modulation of the immune response. *Science*, 186, 307-308.
- Newlin, C. M. and D. L. Gasser (1973). Genetic control of the *in vitro* responses of rat peripheral blood lymphocytes to phytohemagglutinin and concanavalin A. *J. Immunol.* 110, 622-628.
- Pauly, J. L., J. E. Sokal, and T. Han (1973). Whole-blood culture technique for functional studies of lymphocyte reactivity to mitogens, antigens, and homologous lymphocytes. *J. Lab. Clin. Med.* 82, 500-512.
- Phillips, R. D., E. L. Hunt, R. D. Castro, and N. W. King (1975). Thermoregulatory, metabolic, and cardiovascular response of rats to microwaves. *J. Appl. Physiol.* 38, 630-635.

- Prince, J. E., T. H. Mori, J. W. Frazer, and J. C. Mitchell (1972). Cytological aspects of RF radiation in the monkey. *Aerospace Med.* 43, 759-761.
- Riley, V. (1975). Mouse mammary tumors: alterations of incidence as apparent function of stress. *Science*, 189, 465-467.
- Smialowicz, R. J., J. B. Kinn, and J. Elder (1979). Perinatal exposure of rats to 2450-MHz (CW) microwave radiation: effects on lymphocytes. *Radio Sci.* 14 (6S), 147-153.
- Smialowicz, R. J., K. L. Compton, M. M. Riddle, R. R. Rogers, and B. L. Brugnolotti (1980). Microwave radiation (2450 MHz) alters the endotoxin-induced hypothermic response of rats. *Bioelectromagnetics*, 1, 353-361.
- Stobo, J. D. and W. E. Paul (1973). Functional heterogeneity of murine lymphoid cells. *J. Immunol.* 110, 362-375.
- Stodolnik-Baranska, W. (1967). Lymphoblastoid transformation of lymphocytes *in vitro* after microwave irradiation. *Nature*, 214, 102-103.
- Strong, D. M., A. A. Ahmed, J. Sher, R. C. Knudson, and K. W. Sell (1974). Specificity of *in vitro* murine B cell activation by protein and polysaccharide polymers. *J. Immunol.* 113, 1429-1437.
- Thaxton, P. and H. S. Siegel (1973). Modification of high temperature and ACTH induced immunodepression by metyrapone. *Poultry Sci.* 52, 618-624.
- Wallenstein, S., C. L. Zucker, and J. L. Fleiss (1980). Some statistical methods useful in circulation research. *Circulation Res.* 45, 1-9.
- Weil, C. M. (1978). The characteristic impedance of rectangular transmission lines with thin center conductor and air dielectric. *IEEE Trans. Microwave Theory & Techniques*, MTT-26(4), 238-242.
- Weil, C. M., W. T. Joines, and J. B. Kinn (1981). Frequency range of large scale TEM mode rectangular strip lines. *Microwave J.* 24, 93-100.
- Wiktor-Jedrzejczak, W., A. Ahmed, K. W. Sell, P. Czerski, and W. M. Leach (1977). Microwaves induce an increase in the frequency of complement receptor-bearing lymphoid spleen cells in mice. *J. Immunol.* 118, 1499-1502.
- Williams, R. M., M. J. Moore, and B. Benacerraf (1973). Genetic control of thymus-derived cell function. IV. Mitogen responsiveness and mixed lymphocyte reactivity of thymus cells and lymph node cells from Lewis and Brown Norway rats. *J. Immunol.* 111, 1579-1584.